

**Remarks**

Claims 1, 2 and 4-21 are pending in the application. Claims 1, 2, 4-19 and 21 are rejected. Claim 20 is objected to. Claims 1, 12, and 21 are hereby amended.

**Rejections****35 U.S.C. 102 (b) – Garman with support from Rohatgi, Wei, and Tsien**

Claims 1, 3-4, 6-8, 10, 12-13, and 15-18 are rejected under 35 U.S.C. 102(b) as being anticipated by Garman et al. (GB 2278356) (hereinafter “Garman”) with support from Rohatgi et al. (J. Phys. Chem. (6/1966) vol. 70 (6), pages 1695-1701) (hereinafter “Rohatgi”), Wei et al. (Anal. Chem (5/1994), vol. 66 (9), pages 1500-1506) (hereinafter “Wei”), and Tsien (U.S. Pat. No. 5,741,657) (hereinafter “Tsien”).

The Office Action essentially states, in part, that the teachings of Garman, Rohatgi, Wei, and Tsien provide evidence that fluorescein and tetramethylrhodamine (TMR) are capable of dye-stacking such that one skilled in the art would conclude that the fluorescein and TMR attached to Garman’s enzyme substrate inherently undergo dye-stacking in an aqueous buffer.

Applicants have amended claims 1, 12, and 21 to include the limitations (1) that the dye groups are identical, (2) that release, or separation, of the dye groups from dimerization or stacking produces an at least 10-fold increase in fluorescence, and (3) that the emission wavelength for the fluorescence is at or above 570 nm. Support for these amendments appear in the specification, e.g., at (1)p. 8, lines 12-13; (2) p. 14, lines 27-28; and (3) p. 14, line 31 to p. 15, line 10 and p. 18, Table 1, respectively.

“A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” MPEP 2131 (citing Verdegaal Bros. V. Union Oil Co. of California, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)).

Garman does not disclose a structure having identical dye groups that produce an at least 10-fold increase in fluorescence upon release, or separation, of the dye groups from dimerization or stacking at emission wavelengths at or above 570 nm. Because Garman does not disclose expressly, or inherently, even with the support of Rohatgi, Wei, and Tsien, every element of the presently claimed invention, Applicant(s) submit that the cited references cannot support a 35 U.S.C. 102(b) rejection and respectfully requests that the rejection be withdrawn.

35 U.S.C. 103(a) – Garman, Rohatgi, Wei, Tsien, and Komoriya

Claims 1-2, 4-8, and 10-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Garman (GB 2278356) (hereinafter “Garman”) as supported by Rohatgi (J. Phys. Chem (6/1966) vol. 70 (6), pages 1695-1701), Wei (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506), and Tsien (U.S. Pat. No. 5,741,657), and in view of Komoriya (U.S. Pat. No. 5,714,342).

35 U.S.C. 103(a) – Garman, Rohatgi, Wei, Tsien, Komoriya, and Manafi

Claims 9 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Garman (GB 2278356) (hereinafter “Garman”) as supported by Rohatgi (J. Phys. Chem (6/1966) vol. 70 (6), pages 1695-1701), Wei (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506), and Tsien (U.S. Pat. No. 5,741,657), and in view of Komoriya (U.S. Pat. No. 5,714,342) and further in view of Heath, Jr., (U.S. Pat. No. 5,235,039).

35 U.S.C. 103(a) – Garman, Rohatgi, Wei, Tsien, and Manafi

Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Garman (GB 2278356) (hereinafter “Garman”) as supported by Rohatgi (J. Phys. Chem (6/1966) vol. 70 (6), pages 1695-1701), Wei (Anal. Chem. (5/1994), vol. 66 (9), pages 1500-1506), and Tsien (U.S. Pat. No. 5,741,657), and in view of Manafi (Microbiol. Reviews (9/1991), vol. 55(3), pages 335-348).

With regard to the three section 103(a) rejections, the Office Action essentially states, in part, that Garman teaches dimerizing dyes and that it would have been obvious to have combined the teachings of Garman with the other references.

According to MPEP 2142, to establish a case of *prima facie* obviousness, three basic criteria must be met: 1) there must be some suggestion or motivation, either in the references or generally known to one of skill in the art, to modify or combine the reference teachings, 2) there must be reasonable expectation of success, and 3) the prior art references must teach or suggest all the claim limitations. The ability to modify the method of the references is not sufficient. The reference(s) must provide a motivation or reason for making the changes. *Ex parte Chicago Rawhide Manufacturing Co.*, 226 USPQ 438 (PTO Bd. App. 1984).

Applicants respectfully submit that the references cannot support a case of *prima facie* obviousness as to the claims because, among other possible reasons, the cited references do not provide a motivation or suggestion to use identical dyes groups because Garman teaches only

energy transfer. Energy transfer does not occur with identical dye groups because such dyes absorb energy at the same wavelengths, which provides no driving force to cause a transfer of energy.

According to *Ex parte Hartmann*, 186 USPQ 366, 367 (PTO Bd. App. 1974), references cannot be properly combined with each other when such would result in destroying that on which the invention of one of the references is based. Substituting pairs of dyes that would not undergo energy transfer for the dye pairs disclosed in Garman would destroy the invention of Garman, which is stated to be an improved method of preparing a FRET (fluorescence energy transfer) substrate. *See, e.g.*, Garman at p. 2, 3<sup>rd</sup> full paragraph.

The references also do not provide a motivation or suggestion to use only dyes that have emission wavelengths in the visible spectrum at or above 570 nm. This aspect of the invention provides an advantage because it maximizes the signal-to-noise ratio of the system. As explained in the specification, most fluorogenic substrates emit in the far UV wavelength region where most bacteria growth media have significant auto fluorescence. (*See* p. 14, line 31 to p. 15, line 10.) In contrast, the fluorogenic substrates of the present invention have a red-shifted emission wavelength at or above 570 nm, which is outside of the auto fluorescence range of most bacteria.

For these reasons, Applicants submit that the cited references will not support a 103(a) rejection of the claimed invention and request that the rejection be withdrawn.

In addition to the foregoing arguments, Applicants submit that a dependent claim should be considered allowable when its parent claim is allowed. *In re McCain*, 101 USPQ 411 (CCPA 1954). Accordingly, provided independent claims 1, 12 and 21 are allowed, all claims depending therefrom should also be allowed.

### **Objection**

Claim 20 is objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form.

Applicants have not rewritten claim 20 because they believe the parent claim of claim 20 is allowable.

Based on the foregoing, it is submitted that the application is in condition for allowance. Withdrawal of the rejections under 35 USC 102 and 103 is requested. Examination and reconsideration of the claims are requested. Allowance of the claims at an early date is solicited.

Applicants and their attorney thank the Examiner for the telephone interview of November 14, 2002 during which the essence of the amendments and arguments contained herein were discussed. Examiner Marjorie Moran, attorney Melanie Gover, inventor Ai-Ping Wei, and 3M employee Steve Wolf were present at the interview. The Examiner is invited to contact Applicants' attorney if the Examiner believes any remaining questions or issues could be resolved.

Respectfully submitted,

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**Version With Markings to Show Changes Made**

1. (thrice amended) A method of biological assay comprising:

- a) providing an enzyme substrate comprising two or more identical fluorescence dye groups bound to a flexible peptide comprising one or more enzymatically cleavable bonds, the dye groups being drawn together by free energy attractions such that the dye groups self-quench their fluorescence by dye stacking or dimerization, and
- b) contacting said substrate with a substance being assayed to determine the presence of an enzyme capable of cleaving an enzymatically cleavable bond wherein the enzymatic cleaving of said cleavable bond of the peptide will release the fluorescent dye groups from dye stacking or dimerizing, thereby producing an at least 10-fold increase in fluorescence intensity over that of the quenched dye groups [and] thereby indicating the presence of said enzyme, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.

12. (thrice amended) A protease substrate comprising a flexible peptide and including two identical fluorescence dye groups that are drawn together by free energy attractions so as to self-quench fluorescence of the dye groups by intramolecular dimerization or stacking and which, when separated, fluoresce at an at least 10-fold increase in fluorescence intensity over that of the quenched dye groups, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.

21. (thrice amended) An assay method of detecting a microorganism, which microorganism produces a characteristic enzyme, comprising:

- a) providing an enzyme substrate specific for said characteristic enzyme produced by said microorganism comprising two or more identical fluorescence dye groups bound to a flexible peptide comprising one or more bonds cleavable by said characteristic enzyme, the dye groups being drawn together by free energy attractions such that the dye groups self-quench their fluorescence by dye dimerization or stacking, and

- b) cleaving one or more of said cleavable bonds of the peptide by said characteristic enzyme to release the fluorescence dye groups from dye dimerization or stacking, thereby producing an at least 10-fold increase in fluorescence intensity over that of the quenched dye groups thereby [which indicates] indicating the presence of said microorganism, wherein the emission wavelength of the fluorescent dye groups is at or above 570 nm.